Citation:

Benassi-Evans B, Clifton PM, Noakes M, Keogh JB, Fenech M. High protein-high red meat versus high carbohydrate weight loss diets do not differ in effect on genome stability and cell death in lymphocytes of overweight men. *Mutagenesis.* 2009;24(3):271-277.

PubMed ID: 19264840

Study Design:

Randomized Clinical Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To compare high carbohydrate weight loss and high protein-high red meat weight loss diets with different micronutrient and macronutrient profiles on genome stability in peripheral blood lymphocytes.

Inclusion Criteria:

- Males
- 20-65 years old
- BMI 27-40
- At least one other risk factor for cardiovascular disease other than obesity

Exclusion Criteria:

- Those with history of metabolic or coronary disease or type 1 or 2 diabetes.

Description of Study Protocol:

Recruitment Subjects were recruited by public advertisement.

Design Randomized clinical trial

Blinding used (if applicable): implied with laboratory measurements

Intervention (if applicable)

- One of two isocaloric energy-restricted diets which would produce approximate weight loss of 1 kg/week: High protein-red meat diet or High carbohydrate-low red meat.
- Subjects followed diets for 12 weeks

Statistical Analysis

- Two way (mixed between-within subjects) analysis of variance (ANOVA) to compare the main effect for time and diet type and any interaction effect.
- Independent t-tests were used to compare the groups at baseline.
- Significance for all tests was accepted at $P< 0.05$

Data Collection Summary:

Timing of Measurements

- After a 12 week intensive weight loss phase, a 9 month weight maintenance period followed
- Dietary intake for 6 days/month was analyzed
- Subject's weight and weight loss measured at 0,12 and 52 weeks.
- Venous blood was collected and peripheral lymphocyte DNA damage was measured at weeks 0, 12, and 52.

Dependent Variables

- Weight
- Frequency of binucleated cells (BN) with micronuclei (MN) measured by cytokinesis-block micronucleus cytome (CBMN-Cyt) assay. (MN-BN)
- Frequency of binucleated cells with nucleoplasmic bridges (Npb) measured by CBMN-Cyt assay. (Npb-BN)
- Frequency of binucleated cells with nuclear buds (Nbud-BN) measured by CBMN-Cyt assay. (Nbud-BN)
- Total DNA damage calculated as: DNA damage=NM+BN+Npb-BN+Nbud-BN
- Rate of necrosis (marker of cytotoxicity)
- Rate of apoptosis (marker of cytotoxicity)
- Nuclear division index (NDI) calculated as: NDI=(M1+2M2+3M3+4M4)/*N*) where M1 - M4 represent the number of cells with 1-4 nuclei and *N* is the total number of viable cells scored (excluding necrotic and apoptotic cells).

Independent Variables

- High protein-high red meat weight loss diet - (35% protein, 40% carbohydrate, 25% fat). Subject's monthly diet checklists (6 per month) analyzed by FoodWorks software.
- High carbohydrate-low red meat weight loss diet - (17% protein, 58% carbohydrate, 25% fat). Subject's monthly diet checklists (6 per month) analyzed by FoodWorks software.

Control Variables

Description of Actual Data Sample:

Initial N: 33 males

Attrition (final N): 33 males

Age:

- High protein (HP) diet group (n=16) 54.94±1.17 years
- High carbohydrate (HC) diet group (n=17) 52.94±1.50 years

Ethnicity: Australian

Other relevant demographics:

Anthropometrics

- Weight: HP = 99.84±2.45 kg and HC = 99.58±3.61 kg
- BMI (kg/m^2): HP = 32.42±0.79 and HC = 31.47±0.96

Subjects were not significantly different at baseline in terms of age, weight, or BMI.

Location: Australia

Summary of Results:

Key Findings:

- There was no change in MN-BN (DNA damage biomarkers) seen with either time or diet type.
- There was a significant reduction with time (P=0.03) but not dietary pattern for Nbud-BN (DNA damage biomarker).
- Significance was almost met for an increase in time for Npb-BN (DNA damage biomarker).
- When frequency for the three DNA damage biomarkers is combined (total DNA damage) no effect of time or dietary pattern is evident.
- No significant effect with time or diet was found for NDI (nuclear division index) at 0,12 and 52 weeks.
- There was a significant effect of time only for reduced necrosis (P=0.037) and reduced apoptosis (P=0.007).
- A significant increase with time but not dietary pattern was found for plasma folate (P<0.001).
- There was a significant difference between diets with respect to changes in plasma vitamin B12 with time (2-way ANOVA time by diet interaction P=0.01 showing a trend for an increase in plasma vitamin B12 for the High protein-high red meat diet and corresponding decrease for the high carbohydrate-low red meat diet over the 52 week period.

Other Findings

Weight at each stage of the trial

			Week 0	Week 12	Week 52	One-way ANOVA
Weight (kg)	HP	n=16	99.84±2.45^a	90.14±2.27^b	89.15±2.10^b	P<0.001
	HC	n=17	99.58±3.62	90.61±2.96^b	87.34±2.30^b	P<0.001
Values shown as mean ±SEM. Values in one row not sharing a letter are significantly different P < 0.05.						

- Both diets produced an average weight loss (after 12 weeks) of 9.3± 0.7kg with no further change after 52 weeks
- There were no differences in macronutrient intakes between groups, however, there were significantly higher intake levels of folate, calcium, niacin, vitamin E, riboflavin and iron in the high protein-high red meat group but significantly higher intake levels of retinol in the high carbohydrate low red meat group.

Author Conclusion:

Results from this study suggest that a high protein (high red meat) diet does not appear to influence the genome stability profile of peripheral blood lymphocytes differently to a high carbohydrate (low red meat) diet, when assessed using the CBMN-Cyt assay in overweight men who are not folate or vitamin B12 deficient.

Reviewer Comments:

- *No control group in this study*
- *Only males studied*
- *Small numbers of subjects in groups*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes

2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes

5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes

7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	N/A
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	No
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	No